# THE HYALINE ZONE OF THE CENTRIFUGED EGG OF CUMINGIA

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The egg of the lamellibranch mollusk, *Cumingia tellinoides,* has been extensively used as material for studies with the centrifuge, beginning with the classical experiments of Morgan (1908*a,* 1908*b,* 1909, 1910). The results of these investigations indicated that the visible granular substances of the protoplasm, displaceable with centrifugal force, bore no necessary relation to the polarity, symmetry, or organ formation of the developing embryo. Since these embryological studies were made, *Cumingia* eggs have been centrifuged by Heilbrunn to study the changes in protoplasmic viscosity during maturation, fertilization, and cleavage (1920, 1921, 1925); to determine the effects of temperature on the viscosity of protoplasm (1924*a,* 1924*b*); and for measurements of the absolute viscosity of protoplasm (1926). E. N. Harvey has centrifuged these eggs to measure the tension at the surface (1931*a*), and as material for microscope-centrifuge studies (1931*b,* 1932). They were also centrifuged by Costello (1932), to determine the rôle of the granular constituents of the protoplasm in the surface precipitation reaction.

Morgan (1910) describes the appearance of the centrifuged unfertilized egg of *Cumingia* as follows: " When the egg is centrifuged the pigment and yolk are carried to the outer pole, the oil to the inner. Between the two lies a band of clear protoplasm." This description is based on sections as well as on the appearance of the living egg. Of the displaced granular constituents, it is the yolk alone, according to Morgan, which appears in the sections, the pigment and oil being dissolved by the reagents. The broad clear zone is figured as containing finely granular, stainable protoplasm. Morgan (1908*a,* 1908*b,* 1909) had previously described in less detail the three zones produced by centrifuging.

Morgan (1927, p. 501) has amplified the above description as follows: " After centrifuging, the egg shows four zones, similar to those in centrifuged eggs of the sea-urchin. There is (1) a large oil cap; (2) a broad, clear zone; (3) a small yolk field; and (4) a pink, pigmented region opposite to the oil cap."

All other workers have described but three zones. Heilbrunn (1920) says: "To one pole pass the presumably lighter oil globules, to the opposite pole the heavier pigment." Harvey (1931*b*) says: ". . . the oil is stratified in two-thirds the time necessary to stratify the yolk." These workers observed only living material.

The question naturally arises as to whether there are three zones and two types of granules in the centrifuged egg of *Cumingia*, or four zones and three types of granules (assuming the hyaline zone in both cases to be non-granular). It is quite evident that Harvey and Heilbrunn have observed but one type of granule at the centrifugal pole of the egg, and that in their descriptions yolk and pigment are synonymous. It is well known that the pigmentation of these granules may vary from deep pink to an almost colorless condition in different lots of eggs.

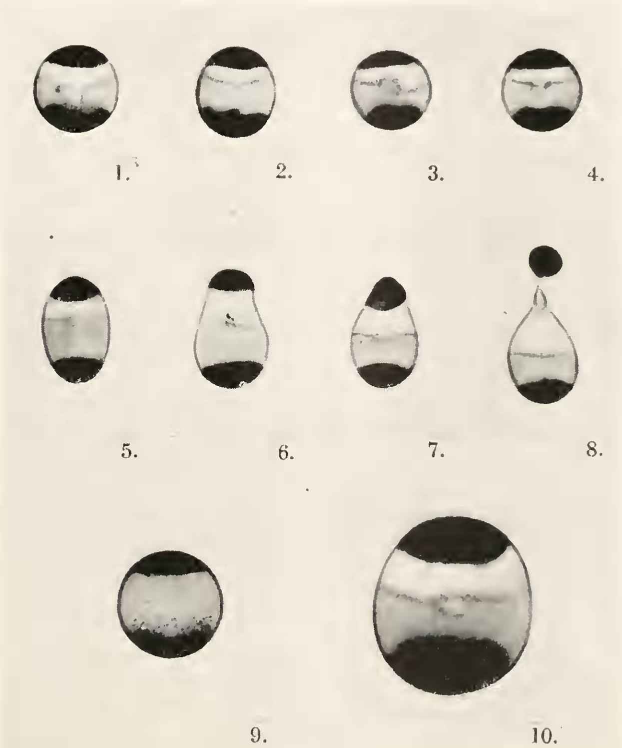
Unfortunately Morgan does not give the centrifugal force developed by the Bausch and Lomb hand centrifuge nor by the water centrifuge which he used in his experiments. From his figures, however, we may be certain that the forces used were small. Heilbrunn (1924*a*) used a force of 311 times gravity and stratified the eggs in 90 seconds or less. Heilbrunn (1925, 1926) used a force of 4,968 times gravity, and stratified the eggs in 5 seconds or less. Harvey (1931*a*) used a force of 6,595 times gravity to pull the eggs apart.

I have been interested in the application of great centrifugal forces for long periods of time. If the eggs are centrifuged for 10 to 20 minutes with a force of about 6,000 times gravity, in hæmatocrit tubes containing a centrifugal layer of isotonic sucrose, a stratification of the egg is produced markedly different from that described above. (See Photographs 1–10). There are visible in the living centrifuged egg the following zones: (1) an oil cap; (2) a clear hyaline zone containing no visible granules; (3) a broad zone of very small colorless granules; and (4) a zone of pink, pigmented granules. Zones (2) and (3) have been produced by separating out the finely granular material from the hyaline protoplasm in the broad, clear zone described by Morgan. The spindle may lie at any angle with respect to the stratification. The spindle generally lies in the zone of small colorless granules in the more strongly centrifuged eggs, or at the border of the hyaline zone and the zone of small granules in the less strongly centrifuged eggs.

Eggs centrifuged at any time after fertilization before the second cleavage may be stratified by prolonged centrifuging to give these same zones, though the duration of time of centrifuging to produce a given end-point varies with the phase of mitosis (see Heilbrunn, 1921).

If the eggs are centrifuged for 30 seconds with a force of 6,000 times gravity, or for a correspondingly longer time at lower forces, only three zones are produced. These are: (1) the oil cap; (2) the broad, clear zone; and (3) the pigmented zone.

The increase in height of the true hyaline zone formed by the movement centrifugalwards of the granules of the broad clear zone may be expressed as a function of the centrifuging time (for a force of about 6,000 times gravity). (See Table I.)



## EXPLANATION OF PHOTOGRAPHS

All photographs are of living unfertilized eggs, arranged with the oil cap uppermost in each case.

PHOTOGRAPHS 1 AND 2. Egg centrifuged 3 minutes, photographed 14 minutes later.

PHOTOGRAPHS 3 AND 4. Egg centrifuged 5 minutes, photographed 12 minutes later. Maturation spindle visible.

PHOTOGRAPH 5. Egg centrifuged 15 minutes, photographed 5 minutes later.

PHOTOGRAPH 6. Egg centrifuged 20 minutes, photographed 4 minutes later.

PHOTOGRAPH 7. Egg centrifuged 30 minutes, photographed 1 minute later.

PHOTOGRAPH 8. Same. The oil cap is pulling off as a spherical fragment.

PHOTOGRAPH 9. Egg centrifuged 1 minute, photographed 30 minutes later.

PHOTOGRAPH 10. Egg centrifuged 10 minutes, photographed 9 minutes later.

With prolonged centrifuging (30 minutes or longer), the oil cap separates as a sphere from the rest of the egg. (See Harvey, 1931*a*.)

In sections of centrifuged eggs fixed by the Champy-Kull technique, sectioned, and stained with Heidenhain's hæmatoxylin, the same four zones of stratification are found. The hyaline zone has coagulated to give a finely granular appearance. The small colorless granules remain, but do not stain intensely by this procedure. The oil globules are preserved, and appear as intensely black spheres at the centripetal pole of the egg. Since the term yolk has not been applied to any specific chemical substance in the eggs of marine invertebrates, the exact terminology to be applied to the pigmented granules seems of little importance.

Heilbrunn (1926) obtained a value of 0.043 poises for the absolute viscosity of *Cumingia* egg protoplasm. This value is based on the assumption that the pigmented granules were moving through hyaline or

Table I

| Centrifuging time in minutes | Height of hyaline zone in fractions of egg axis | Height of zone of small granules in fractions of egg axis |
|---|---|---|
| 1/2 | 0 | 1/2 |
| 1 | 0 | 1/2 |
| 3 | 1/16 | 7/16 |
| 5 | 1/8 | 3/8 |
| 10 | 1/4 | 1/4 |
| 20 | 3/10 (egg pear-shaped) | 3/10 |
| 30 | 2/5 (oil cap pulling off) | 1/5 |

granule-free protoplasm. Actually, they were moving through the finely granular protoplasm of the broad clear zone. Therefore the absolute viscosity of the hyaline protoplasm would be somewhat lower than this figure. However, Harvey (1931*b*) gives a value for the average diameter of the pigment granules as 0.5 $\mu$. My own measurements of granules in fixed preparations tend to verify this value, although measurements of such small particles are never very certain. Neglecting the Cunningham correction, this would give the suspension of the broad clear zone a viscosity of 0.118 instead of 0.043 poises. Several equations have been derived for estimating the viscosity of the dispersion medium when the viscosity of the suspension and the volume concentration of the suspended particles are known. For the egg of *Cumingia*. the volume of the zone occupied by the small colorless granules is about equal to the volume of the true hyaline zone. If this zone consisted of packed spherical granules of uniform diameter, they would occupy approximately 74 per cent of the total volume of this zone. However, there is probably considerable variation in granule size, and it is not certain how much packing occurs. As an approximation we may take the relative volume occupied by the granules in the broad clear zone as 37 per cent. The concentration of granules at which the viscosity is infinity is assumed to be 74 per cent, though this is probably too high. The equations for the viscosity of a suspension, with the values obtained for the viscosity of the hyaline protoplasm of the *Cumingia* egg, by the use of each, are listed below:

(1) $\eta_s = \eta \dfrac{1 + 0.5f}{(1-f)^4}$ (Einstein, from Kunitz, 1926) $\eta = 0.016$

(2) $\eta_s = \eta(1 + 4.5f)$ (Hatschek, 1910) $\eta = 0.044$

(3) $\eta_s = \eta \dfrac{1}{1 - af}$ (Hess, 1920)

(3a) $\eta_s = \eta \dfrac{1}{1 - \sqrt[3]{f}}$ (Hatschek, 1911) $\eta = 0.033$

(4) $\eta_s = \eta \dfrac{1}{(1 - f - 3/2f^{5/3})^{5/2}}$ (Smoluchowski, 1916) $\eta = 0.008$

(5) $\eta_s = \eta \dfrac{1}{\left(1 - \dfrac{f}{f_\infty}\right)}$ (Bingham and Durham, 1911) $\eta = 0.059$

where $\eta_s$ is the viscosity of the suspension (0.118), $\eta$ is the viscosity of the dispersion medium, $f$ is the ratio of the volume of suspended particles to the total volume of the suspension (37 per cent), $f_\infty$ is the value of $f$ at which viscosity becomes infinite (74 per cent), and $a$ is a constant.

As Kunitz points out, equation (1) is purely empirical. Equation (2) may be applied in cases where $f$ is large. Equation (3), involving the constant $a$, cannot be applied except by assuming

$$a = \frac{1}{\sqrt[3]{f^2}} (= 1.94),$$

which makes this equation identical with one previously derived by Hatschek (3 $a$) for very concentrated suspensions (> 50 per cent) of deformable particles. Hess has applied this equation to suspensions of both low and high concentration. Equations (4) and (5) are also empirical. It is not possible to state at present which of these equations applies best to this particular case, so the values calculated are indicative only of the order of magnitude of the viscosity of the hyaline protoplasm. Obviously, any value lower than the viscosity of water (0.01 poises) is an impossible figure.

Completely stratified *Cumingia* eggs were crushed in the manner described by Costello (1932) and the presence or absence of a surface precipitation reaction observed for the different zones of stratification. This was done to determine whether or not the small colorless granules are directly involved in the surface precipitation reaction, as Heilbrunn has previously shown for the pigment granules of the *Arbacia* egg. It was usually possible to obtain a surface precipitation reaction from the heavy pole of the egg, if the egg were gently ruptured. From protoplasm extruded at the light pole, however, a definite membrane was never obtained. Whether this is due to the greater fluidity of the protoplasm at this pole, or due to the absence of a particular type of granule, could not be determined. Since the granules of the zone of small colorless granules are about at the limits of microscopical vision (0.2 $\mu$ or less in diameter), it was not possible to determine whether or not they disintegrated during the formation of the surface precipitation membrane at the heavy pole of the egg.

Considering the results outlined above, Morgan's (1910) analysis of the relation between organ-forming substances and embryonic localization in the egg of *Cumingia* is somewhat incomplete, since it does not preclude the possibility of almost normal distribution of the small colorless granules of the broad clear zone to all of the early blastomeres. (The small colorless granules are of such a size that they could occupy the interspaces between the packed pigment granules or oil in the eggs centrifuged with the forces used by Morgan.) However, considering the results of the centrifuge experiments on other eggs, it seems improbable that the small colorless granules are any more morphogenic than the other types of granules. (But compare the results of Conklin, 1931, obtained by prolonged centrifuging of the egg of *Styela*.)

## Summary

1. The zones of stratification of the centrifuged egg of *Cumingia* have been redescribed.

2. The relations of the presence of granules in the "broad clear zone" to previous work on (*a*) absolute protoplasmic viscosity, (*b*) the surface precipitation reaction, and (*c*) embryonic localization have been discussed.

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